National Toxicology Program
Toxicity Report Series
Number 71

NTP Technical Report on the Toxicity Studies of

Malachite Green Chloride and Leucomalachite Green

(CAS Nos. 569-64-2 and 129-73-7)

Administered in Feed to F344/N Rats and B6C3F$_1$ Mice

June 2004
NIH Publication No. 04-4416

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration (FDA); and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Toxicity Study Report were performed under the direction of the NCTR and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals.

These studies are designed and conducted to characterize and evaluate the toxicologic potential of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Toxicity Study Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's toxic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Toxicity Study Reports and full versions of the most recent reports and other publications are available from the NIEHS's Environmental Health Perspectives (EHP) http://ehp.niehs.nih.gov (866-541-3841or 919-653-2590). In addition, printed copies of these reports are available from EHP as supplies last. A listing of all the NTP Toxicity Study Reports printed since 1991 appears on the inside back cover.

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The studies on malachite green chloride and leucomalachite green were conducted at the FDA's National Center for Toxicological Research under an interagency agreement between the FDA and the NIEHS. The studies were designed and monitored by a Toxicology Study Selection and Review Committee composed of representatives from the NCTR and other FDA product centers, NIEHS, and other *ad hoc* members from other government agencies and academia. The interagency agreement was designed to use the staff and facilities of the NCTR in the testing of FDA priority chemicals and to provide FDA scientists and regulatory policymakers information for hazard identification and risk assessment.

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PEER REVIEW

The draft report on the toxicity studies of malachite green chloride and leucomalachite green was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the Toxicity Study Report presents the experimental results and conclusions fully and clearly.

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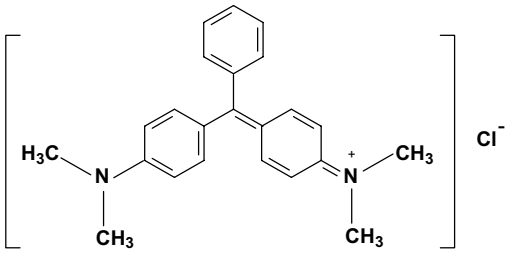
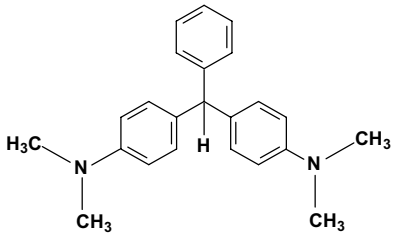
ABSTRACT



MALACHITE GREEN CHLORIDE

CAS No. 569-64-2

Chemical Formula: $C_{23}H_{25}ClN_2$ Molecular Weight: 364.92



LEUCOMALACHITE GREEN

CAS No. 129-73-7

Chemical Formula: $C_{23}H_{26}N_2$ Molecular Weight: 330.47

Malachite Green Chloride

Synonyms: *bis*[*p*-(Dimethylamino)phenyl]phenylmethylium chloride; *N*-[4-[[4-(dimethylamino)-phenyl]phenylmethylene]-2,5-cyclohexadien-1-ylidene]-*N*-methylmethanaminium chloride

Trade names: Aniline Green; Benzal Green; Benzaldehyde Green; China Green; C.I. Basic Green 4; C.I. 42000; Diamond Green B; Diamond Green Bx; Diamond Green P Extra; Fast Green; Light Green N; New Victoria Green Extra I; New Victoria Green Extra II; New Victoria Green Extra O; Solid Green O; Victoria Green B; Victoria Green WB

Leucomalachite Green

Synonym: *p,p′*-Benzylidenebis-*N,N*-dimethylaniline

Malachite green chloride is a triphenylmethane dye used in the fish and dye industries. Leucomalachite green is prepared by the reduction of malachite green chloride. Malachite green chloride was nominated for toxicity and carcinogenicity testing by the Food and Drug Administration and selected by the National Institutes of Environmental Health Sciences for carcinogenicity testing by the National Toxicology Program (NTP) due to the potential for significant worker and consumer exposure and lack of carcinogenicity data. The current 28-day studies were conducted as part of an overall effort by the NTP to determine the toxicity and carcinogenicity of malachite green chloride.

Male and female F344/N Nctr BR rats and B6C3F$_1$/Nctr BR (C57BL/6N × C3H/HeN MTV$^-$) mice were exposed to malachite green chloride (95% pure) or leucomalachite green (99% pure) (male rats and female mice only) in feed for 28 days. Animals were evaluated for clinical pathology and histopathology. Genetic toxicity studies for malachite green chloride were conducted *in vitro* in *Salmonella typhimurium* and *in vivo* in rat bone marrow

erythrocytes and in mouse peripheral blood erythrocytes. Genetic toxicity studies for leucomalachite green were conducted *in vivo* in mouse peripheral blood erythrocytes.

Groups of eight male and eight female rats and mice were fed diets containing 0, 25, 100, 300, 600, or 1,200 ppm malachite green chloride for 28 days. Additional groups of eight male and eight female rats designated for thyroid hormone assays were fed diets containing 0 or 1,200 ppm malachite green chloride. Groups of eight male rats and eight female mice were fed diets containing 0, 290, 580, or 1,160 ppm leucomalachite green for 28 days. Additional groups of eight male rats designated for thyroid hormone assays were fed diets containing 0 or 1,160 ppm leucomalachite green.

All rats and mice survived to the end of the studies. In the malachite green chloride study, the body weight gain of males rats in the 1,200 ppm group was significantly less than that of the controls. The final mean body weight of female rats and mice in the 1,200 ppm groups and the body weight gains of female rats and mice in the 600 (rats only) and 1,200 ppm groups were significantly less than those of the controls. In the leucomalachite green study, the final mean body weight of male rats and female mice in the 1,160 ppm groups and the mean body weight gains of male rats and female mice in the 580 and 1,160 ppm groups were significantly less than those of the control groups.

In the malachite green chloride study, feed consumption by all exposed groups of male and female rats and mice was generally similar to that by the control groups. Exposure concentrations of 25, 100, 300, 600, and 1,200 ppm resulted in average daily doses of 3 to 190 mg malachite green chloride/kg body weight to male and female rats and 5 to 250 mg/kg to male and female mice. In the leucomalachite green study, feed consumption by all groups of exposed male rats was similar to that by the controls. Dietary concentrations of 290, 580, and 1,160 ppm resulted in average daily doses of approximately 30, 60, and 115 mg leucomalachite green/kg body weight to male rats and approximately 62, 110, and 220 mg/kg to female mice.

In female rats exposed to malachite green chloride, there was a significant increases in γ-glutamyltransferase activities with an activity in 1,200 ppm females seven times greater than that in the controls. Likewise, γ-glutamyltransferase activity in male rats exposed to 1,160 ppm leucomalachite green was twice that in the controls. On days 4 and 21, the concentration of thyroxine was significantly decreased in male rats exposed to 1,160 ppm leucomalachite green and the concentration of thyroid-stimulating hormone was significantly increased.

In the malachite green chloride study, the relative liver weights of 600 and 1,200 ppm male rats and the relative and absolute liver weights of 300 ppm or greater female rats were generally significantly greater than those of the controls. In the leucomalachite green study, the relative liver weights of 290 ppm or greater male rats were significantly greater than those of the control group.

No gross lesions were observed in rats or mice and no microscopic lesions were observed in female mice that were attributed to malachite green chloride exposure. Microscopically, the incidences of hepatocyte cytoplasmic vacuolization were significantly increased in 1,200 ppm male and female rats exposed to malachite green chloride. No gross lesions were observed in rats or mice that could be attributed to leucomalachite green exposure. Microscopically, the incidences of hepatocyte cytoplasmic vacuolization were significantly increased in 580 and 1,160 ppm male rats. The incidence of multifocal apoptosis in the transitory epithelium of the urinary bladder was significantly increased in 1,160 ppm female mice exposed to leucomalachite green.

Malachite green chloride, tested at concentrations of 0.1 to 10 μg/plate, was not mutagenic in any of several strains of *Salmonella typhimurium*, with or without S9 metabolic activation. Negative results were also obtained in two *in vivo* micronucleus tests, one that assessed induction of micronuclei in rat bone marrow erythrocytes after three intraperitoneal injections of malachite green chloride, and a second study that determined the level of micronuclei in circulating erythrocytes of male and female mice following 28 days of exposure to malachite green chloride via dosed feed. The frequency of micronucleated normochromatic erythrocytes in peripheral blood was significantly increased in female mice exposed to leucomalachite green in feed for 28 days; no significant increases in micronucleus frequencies were observed in the polychromatic erythrocyte population.